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EFFECT OF QUEEN MANDIBULAR PHEROMONES ON *APIS MELLIFERA* WORKER STINGING BEHAVIOR (HYMENOPTERA: APIDAE)

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Abstract.—Honey bee queen mandibular complex (HQMC), a five component pheromone blend, moderates the stinging behavior of worker honey bees when the workers are exposed to an atmosphere that also contains a queen. If either HQMC or an atmosphere in contact with a queen were absent, laboratory bioassays demonstrated that worker honey bees rapidly became more prone to sting.

The stinging of worker honey bees (*Apis mellifera* L.) is an often-suicidal form of colony defense. Pheromonal enhancement of defensive behavior by numerous alarm pheromone components released by disturbed worker bees has been well documented (see review in Free, 1987). This research deals with a different pheromonal input into the willingness of worker bees to sting: the moderating influence of recently identified queen mandibular gland pheromone components.

Honey bee queen mandibular complex (HQMC) is a five component blend identified by Slessor et al. (1988). HQMC is involved in the formation of worker retinues about the queen, in the inhibition of queen rearing, and in the attraction of worker bees during swarm cluster formation (Slessor et al., 1988; Winston et al., 1989, 1990). The five component blend is more pheromonally active than any of its components or blends of less than five components. Live queens show greater pheromonal activity than either synthetic HQMC or mandibular gland extracts, indicating that HQMC normally acts in concert with non-mandibular gland queen pheromones or queen behavior patterns (Slessor et al., 1988, 1989; Kaminski et al., 1989; Winston et al., 1989, 1990).

MATERIALS AND METHODS

The first experiment examined the influence of HQMC on stinging behavior in the presence of possible volatile queen pheromones. Groups of worker bees were shaken from frames into three holding boxes. Each holding box measured 23 cm high, 17.5 cm wide, and 30.5 cm deep, and had vertical walls of wire mesh for ventilation, and a revolving plexiglass door that allowed one bee at a time to be removed. Three holding boxes were filled. One box contained a colony's queen and a group of workers. The second box contained workers and a cotton wick impregnated with HQMC that was accessible to them. The third box contained workers and a cotton wick containing solvent alone that was accessible to them. This experiment was conducted in a double-blind fashion; researchers conducting the behavioral assays were not informed of which type of wick was which. The HQMC wick contained 10 queen equivalents of HQMC. Ten queen equivalents of HQMC consists of: 2.5 mg

of 9-keto-2(E)-decenoic acid; 1.5 mg of 9-hydroxy-2(E)-decenoic acid (75% R(-)); 0.2 mg of methyl *p*-hydroxy-benzoate; 0.02 mg of 4-hydroxy-3-methoxyphenylethanol. All holding boxes were equipped with a jar of 1:3 (sugar : water) feeding solution that the bees could drink from freely. Approximately 1,500 bees were placed in each box. Bees taken from three colonies were tested sequentially in the manner described, with bees from only one colony being used at any one time.

The three holding boxes were removed from the apiary to a laboratory and housed together in a Conviron CMP 3023 growth chamber. The boxes were separated from physical contact but shared the same atmosphere. The environment of the holding boxes was maintained at 25°C, 99% relative humidity, and a 12L:12D photoperiod regime.

In the second experiment the influence of HQMC on the stinging behavior of workers in the absence of possible volatile queen pheromones was assessed. The procedure for collecting and housing bees was exactly as in the first experiment, except that only two holding boxes of bees were collected from a colony. One box contained workers and a HQMC wick, the other holding box contained workers and a blank wick. Bees taken from three colonies were tested sequentially in the manner described.

Workers were assessed for their willingness to sting 1½ hours, 1 day, and 2 days after removal from their colony. Fifteen bees from each holding box were tested each day. A threshold voltage bioassay described in detail elsewhere (Kolmes and Fergusson-Kolmes, 1989a, b; Echazarreta et al., 1989) was used to measure the threshold of excitation required to elicit stinging in individual bees. A total of 225 bees were tested using this procedure. Data were analyzed using Wilcoxon-Mann-Whitney tests corrected for ties as appropriate.

RESULTS AND CONCLUSIONS

In the presence of volatile queen pheromones (experiment 1) HQMC had an influence upon the stinging threshold voltage of worker bees. After having been removed from the hive for 1½ hours, the workers in the box containing their queen had a significantly higher threshold voltage than the workers in the box containing a blank control wick ($W = 2325.5$, $p = 0.025$). The workers in the box containing the HQMC wick had an intermediate threshold voltage that differed neither from the workers in the box containing the queen ($W = 2193.5$, $p = 0.240$) nor from the bees in the presence of a blank control wick ($W = 2202$, $p = 0.214$) (Fig. 1).

After the bees had been housed in the laboratory for one day, the moderating influence of HQMC on stinging behavior was greater. The workers in the presence of their queen were once again statistically indistinguishable from those in the presence of the HQMC wick ($W = 1979.5$, $p = 0.586$) while workers in the presence of a blank control wick had significantly lower threshold voltages than either workers in the presence of the HQMC wick ($W = 2382.0$, $p = 0.007$) or workers in the presence of their queen ($W = 2307.5$, $p = 0.036$) (Fig. 1).

After the bees had been housed in the laboratory for two days, the influences of the HQMC wick and the queen upon stinging behavior had both faded. The intermediate HQMC group differed from neither the queen group ($W = 2162.5$, $p = 0.356$) nor the blank control wick group ($W = 2154$, $p = 0.390$). The blank control wick group were most easily excited to sting, and they differed in a nearly statistically

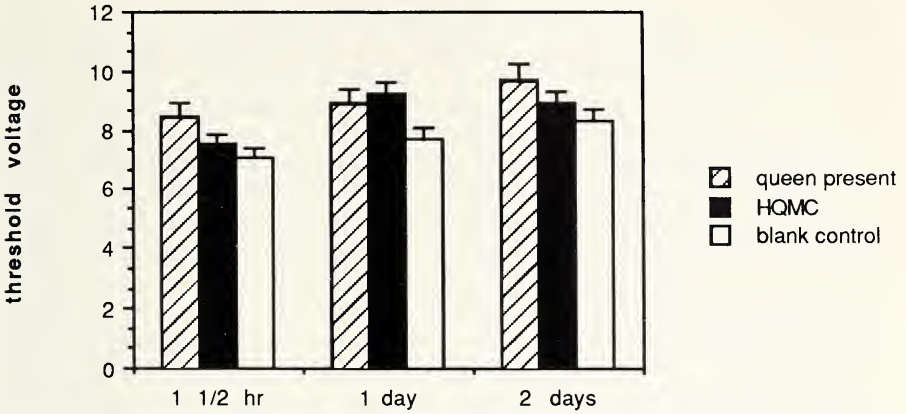

Fig. 1. Threshold voltages for worker bees in holding boxes with their queen, a wick containing HQMC, or a blank control wick. Values are expressed as means, with standard errors of the mean indicated.

significant fashion from the highest threshold voltage group (those bees in the presence of the queen) ($W = 2278.5$, $p = 0.063$) (Fig. 1). It is likely that some components (the aromatics) in the HQMC wick were being depleted.

In the absence of possible volatile queen pheromones (experiment 2) HQMC had no influence at any time upon the stinging threshold voltage of worker bees. This was true when the HQMC wick group was compared to the blank control wick group after 1½ hr in the laboratory ($W = 1999.0$, $p = 0.698$). There was no significant difference between the HQMC wick group and the blank control wick group after one day in the laboratory ($W = 2078.0$, $p = 0.809$). There was also no significant difference between the HQMC wick group and the blank control wick group after two days in the laboratory ($W = 2017.0$, $p = 0.809$) (Fig. 2).

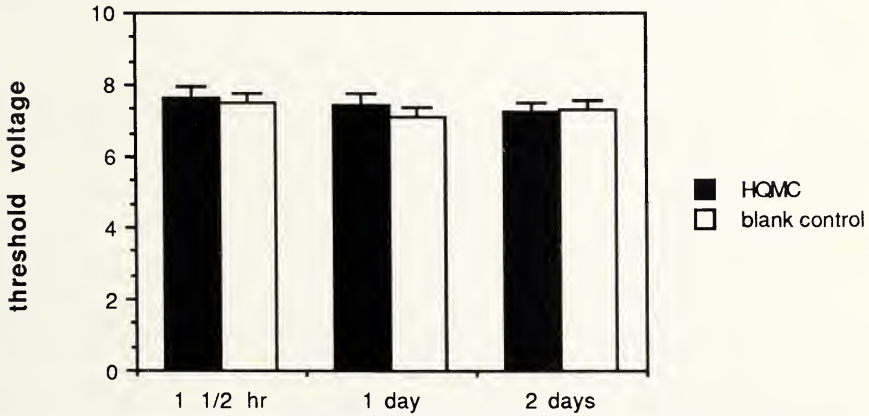

Fig. 2. Threshold voltages for worker bees in holding boxes with a wick containing HQMC or a blank control wick. Values are expressed as means, with standard errors of the mean indicated.

Worker bees responded very rapidly to queen pheromone deprivation with a lowered threshold for eliciting stinging. The effect of HQMC is probably dependent upon the presence of volatile queen pheromones; in the second experiment with the absence of a queen in the growth chamber the effect of a wick containing HQMC was indistinguishable from that of a blank control wick (Fig. 2). It is unlikely that the presence of a queen (first experiment) influenced worker behavior through any increase in the concentrations of the more volatile HQMC components. The workers in the HQMC wick box were already exposed to unusually high HQMC levels, and the workers in the blank control wick box did not behave as though they were being exposed to HQMC.

The significantly higher threshold voltage exhibited after one day by the HQMC wick bees compared with the blank control wick workers is intriguing (Fig. 1). It may be that supernormal levels of HQMC could be used to moderate the tendency of queenless packages of worker bees to sting, as long as one queen or some other source of volatile queen pheromones was present.

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